# Analysis of Cell Types in Continuous Cell Lines and Analysis of the Epidermal Growth Factor Receptor Pathway in Drosophila

A Senior Honors Thesis

Presented in Partial Fulfillment of the Requirements for graduation *with research distinction* in Molecular Genetics in the undergraduate colleges of The Ohio State University

by

Sharon Truesdell

The Ohio State University
June 2009

Project Advisor: Dr. Amanda Simcox, Department of Molecular Genetics

## Abstract

During my research I completed work on two separate projects. First, I worked with cell lines generated from *Drosophila melanogaster* embryos and characterized them for specific cell type. Our lab has recently developed a new method of efficiently creating new Drosophila cell lines. With this approach, many new lines have been created, and I screened them with immunostaining for specific cell type. Out of the 24 lines examined, I found examples of nerve, blood, and epithelial cell types. The epithelial line may be useful for future analysis of the Egfr pathway through *in vitro* studies. Second, I analyzed the Epidermal Growth Factor Receptor (Egfr) Pathway using the Drosophila wing as a model system. Our lab has identified numerous potential targets of the pathway, including *Sulfated (Sulf1*), a 6-O-endosulfatase that modifies glucosamine residues in heparan-sulfated proteoglycans. The role of *Sufl1* and its interactions with the Egfr pathway ligand, Vein, were analyzed genetically. In this thesis, these two projects will be treated as two separate sections.

## Acknowledgements

I would first and foremost like to thank my project advisor, Dr. Amanda Simcox. I am extremely grateful for her guidance, stimulating suggestions and continuous support and encouragement, without which, this thesis would not have been possible. I would also like to thank Dr. Susan Cole and Dr. Paul Fuerst for being apart of my thesis committee and for providing valuable comments. Finally, I would like to thank my family and friends for their love and support throughout my entire research experience.

## Vita

November 11, 1986. . . . . Born- Ashland, Ohio

2005. . . . . Graduated from Ashland High School, Ashland, Ohio

2005- Present. . . . . Undergraduate Student
Department of Molecular Genetics
The Ohio State University

## Publications

Simcox, A., Mitra, S., Truesdell, S., Paul, L., Chen, T., Butchar, J. P., Justiniano, S. (2008). Efficient Genetic Method for Establishing Drosophila Cell Lines Unlocks the Potential to Create Lines of Specific Genotypes. *PloS Genet,* 4(8): e1000142. Doi:10.1371/journal.pgen.1000142.

# Table of Contents

# Chapter 1

## Analysis of Cell Types in Continuous Drosophila Cell Lines

### Introduction

Cell culture has proven to be an extremely useful resource in research for analyzing biological functions and biochemical pathways. Its utilization has lead to a better understanding of countless biological mechanisms and serves as an excellent complement to *in vivo* studies (for example, Banker and Cowan, 1977; Meuillet et al., 2000; Howlin et al., 2008). Although cell culture has great potential, the creation of cell lines in Drosophila often proves difficult, and many potential lines fail to reach an immortal state. Advances in mammalian cell culture have lead to effective methods for establishing and maintaining cell lines. For instance, a technique often employed to maintain cells in an immortal state involves the activation of telomerase by expression of the human telomerase reverse transcriptase (hTERT) gene (for example, Hunag et al., 2007; Li et al., 2007) and the inhibition of tumor suppressors such as p53 and Rb with large T antigen (for example, Foddis et al., 2002; Yang et al., 2007).

In contrast to mammalian cell culture, the development of Drosophila cell culture lags behind. One reason for this is that researchers have lacked an efficient method of creating new lines. Previously, Drosophila cell lines were obtained from primary cultures that spontaneously adopted an immortal cell fate (Schneider, 1972; Debec, 1978). With the use of this approach, however, continuous cell lines were typically only produced from about one out of every ten cultures, and the process takes a long time (Simcox et al., 1985).

Our lab has developed a new method of efficiently producing Drosophila cell lines. Expression of the conserved oncogene $Ras^{V12}$, a constitutively active form of Ras, significantly

increases the percent of cultures that result in continuous cell lines and greatly decreases the time required to establish the lines (Simcox et al., 2008a). UAS-regulated constructs of the oncogene and the green fluorescent protein (GFP) were induced by expression of *Act5C-Gal4*. Act5C, a cytoplasmic actin, drives the expression of Gal4, and as a result, also drives the expression of the UAS transgenes. In addition to efficiently producing new cell lines, this method also allows for the generation of cell lines with a specific genotype. In order to demonstrate this, cell lines were produced in which an RNAi transgene was used to silence the expression of *warts (wts)*, a tumor suppressor gene. Additionally, cell lines were created that contained a loss of function mutant form of the gene *rumi*, which encodes an O-glycosyltransferase and is required for Notch signaling in Drosophila (Acar et al., 2008; Simcox et al., 2008b).

With the use of this method many cell lines were produced including: 3 control lines (*Act5C-Gal4; UAS-GFP*), 8 $Ras^{V12}$ lines (*Act5C-Gal4; UAS-GFP, UAS-*$Ras^{V12}$), 8 $Ras^{V12}$*;* $wts^{RNAi}$ lines (*Act5C-Gal4; UAS-GFP, UAS-*$Ras^{V12}$*, UAS-*$wts^{RNAi}$) , and 5 $Ras^{V12}$*;* $rumi^{-}$ (*Act5C-Gal4; UAS-*$RasV^{12}$*, rumi*$^{-}$). Within these cell lines, the majority of the cells had a spindle-shaped morphology; however, one control cell line was composed of circular shaped cells, and one $Ras^{V12}$*;* $wts^{RNAi}$ line had an epithelial-like morphology. The ability to produce Drosophila cell lines with a specific cell type would prove to be extremely valuable and would significantly advance the field. In order to determine if this method yields cell lines of specific cell types, immunostaining was used to screen the cell lines with four cell specific antibodies to test for the presence of nerve (HRP) (Jackson immunoresearch ), muscle (dMef2) (Lilly et al., 1995), blood (H2) (Kurucz et al., 2003), and epithelial (D- E Cadherin) (Hybridoma Bank, Iowa) cell types.

**Methods**

24 different cell lines were screened, which consisted of 3 control lines (*Act5C-Gal4; UAS-GFP*), 8 $Ras^{V12}$ lines (*Act5C-Gal4; UAS-GFP, UAS-*$Ras^{V12}$), 8 $Ras^{V12}$*; *$wts^{RNAi}$ lines (*Act5C-Gal4; UAS-GFP, UAS-*$Ras^{V12}$*, UAS-*$wts^{RNAi}$) , and 5 $Ras^{V12}$*; rumi*$^{-}$ (*Act5C-Gal4; UAS-*$RasV^{12}$*, rumi*$^{-}$). Cells were plated on coverslips in multi-well cell culture plates or in multi-well slide chambers and were allowed to adhere for 15 minutes to 24 hours. Cells were washed twice with 1X PBS for 30 seconds, fixed with 4% paraformaldehyde in PBS for 20 minutes at room temperature, and then washed three times in 1X PBS for three minutes each. Blocking solution (0.1% Triton X-100, 1% BSA, 1X PBS) was added for 15 minutes to block and permeabilize the cells. Cells were incubated with primary antibody in blocking solution for 1 hour at room temperature. Cells were washed three times in 1X PBS for 4 minutes and Rhodamine conjugated secondary antibodies (1:200) were added for 30 minutes at room temperature. Cells were washed three times in 1X PBS for 4 minutes and mounted using Vectashield (Vector Laboratories). Cells were photographed using a compound fluorescence microscope or a Zeiss 510 META Laser Scanning Confocal microscope by Litty Paul. The following antibodies were used: HRP- Jackson immunoresearch (Rhodamine conjugated) 1:200, dMef2 (Rabbit) 1:500 (Lilly et al., 1995), H2 antibody (Mouse) 1:10 (Kurucz et al., 2003), D- E Cadherin (Rat) 1:5 (Hybridoma Bank, Iowa). All of the secondary antibodies were from Jackson ImmunoResearch.

**Results and Discussion**

Immunostaining was used to analyze the cell type of 24 different cell lines. These cell lines included 3 control lines, 8 $Ras^{V12}$ lines, 8 $Ras^{V12}$; $wts^{RNAi}$ lines, and 5 $Ras^{V12}$; $rumi^{-}$ lines. The cell lines were screened with four different antibodies: HRP, dMef2, H2, and D E-Cadherin. As predicted by the abundant spindle-shaped morphology, many of the cell lines remain in a presumably undifferentiated state; however, a few lines positive for cell markers were observed (Table 1).

The HRP antibody tests for the presence of nerve cells within the cell cultures by binding to the terminal end of neurons, where it is subsequently transported back to the soma (cell body). Positive results with the HRP antibody were observed in 3 cell lines: 1wc, $Ras^{V12}$; $rumi^{-}$ line 1, $Ras^{V12}$; $rumi^{-}$ line 4, and $Ras^{V12}$; $rumi^{-}$ line 8 (Table1). The control line 1wc contained some cells that tested positive with HRP staining; however, the majority of the cells within the culture had negative results. Most of the cells in this line have a circular shape morphology, which is inconsistent with differentiated nerve cells. Three lines of the $Ras^{V12}$; $rumi^{-}$ cells also tested positive for HRP, including $Ras^{V12}$; $rumi^{-}$ line 4 (Figure 1B), and a large percentage of the cells within these cultures were positive for the marker. Activation of the Notch signaling pathway requires the expression of *rumi*, which transfers glucose molecules onto the extracellular domain of the Notch protein, leading to the cleavage of the extracelluar domain. In loss of function mutants of *rumi*, the Notch protein remains in an inactivated state within the cellular membrane (Stanley, 2008). Furthermore, Drosophila embryos that lack Notch signaling have been shown to have an over proliferation of neural tissue (Korochkin et al., 1991). In light of this, it is not too surprising that some of the $Ras^{V12}$; $rumi^{-}$ cell lines were positive for the neural cell marker.

| | HRP | dMef2 | H2 | E-Cad |
|---|---|---|---|---|
| 1wc | + | + | - | - |
| 8wc | - | + | - | - |
| c7 | - | + | - | - |
| Ras1 | - | + | - | - |
| Ras6 | - | + | - | - |
| Ras7 | - | + | - | - |
| Ras8 | - | + | + | - |
| Ras9 | - | + | - | - |
| Ras10 | - | + | - | - |
| Ras11 | - | + | - | - |
| Ras13 | - | + | - | - |
| Ras wts1 | - | + | - | - |
| Ras wts2 | - | + | - | - |
| Ras wts3 | - | + | - | - |
| Ras wts4 | - | + | - | - |
| Ras wts5 | - | + | - | - |
| Ras wts6 | - | + | - | - |
| Ras wts10 | - | + | - | - |
| Ras wtsE | ND* | + | ND* | + |
| Ras rumi1 | + | + | ND* | ND* |
| Ras rumi2 | - | + | ND* | ND* |
| Ras rumi4 | + | ND* | ND* | ND* |
| Ras rumi5 | - | + | ND* | ND* |
| Ras rumi8 | + | + | ND* | ND* |

**Table 1. Results of immunostaining cell lines.** 3 control (1wc, 8wc, and c7), 8 $Ras^{V12}$, 8 $Ras^{V12}$; $wts^{RNAi}$, and 5 $Ras^{V12}$; $rumi^{-}$ cell lines were stained with four antibodies that test for the presence of nerve (HRP), muscle (dMef2), blood (H2), and epithelial (D E-Cadherin) cell types.
*ND not determined. Cell line was not stained with the given antibody.

The dMef2 antibody tests for the presence of the Drosophila myocyte enhancer binding factor-2, which is a transcription factor expressed in skeletal, cardiac, and smooth muscle cell lineages of Drosophila embryos. In early embryos, dMef2 is expressed in mesodermal tissue, but in later embryos, expression is limited to the somatic muscle tissue. Because of this, dMef2 serves as both as a muscle tissue and mesodermal tissue marker (Lilly et al., 1995). Somewhat surprisingly, every cell line tested showed positive results when stained with the dMef2 antibody,

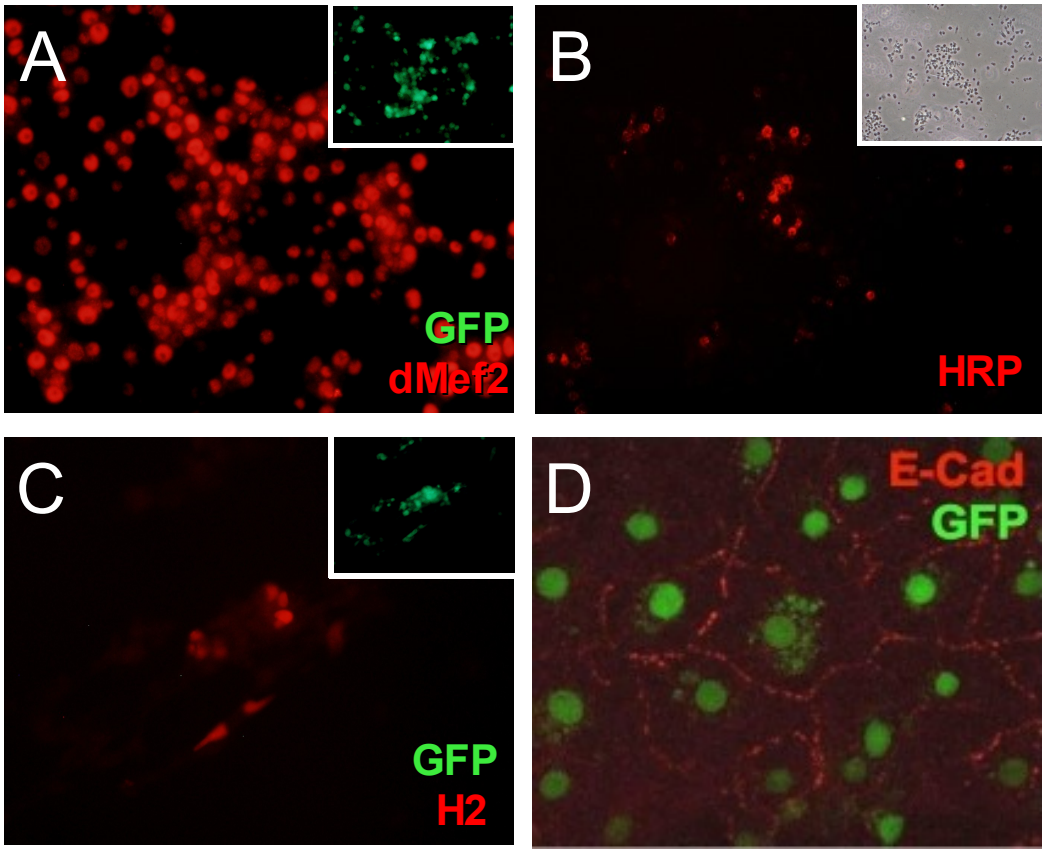



**Figure 1. Cell lines show positive results with immunostaining.** (A) $Ras^{V12}$ line 10 expresses dMef2 suggesting that it is of mesodermal origin. (B) *$Ras^{V12}$; rumi$^{-}$* line 4 expresses HRP indcating the cells express the neural marker. (C) $Ras^{V12}$ line 8 expresses H2 indicating that it may be a blood cell type. (D) Confocal image of $Ras^{V12}$; $wts^{RNAi}$ line E cells that have an epithelial-like morphology and expresses D E-Cadherin.

which indicates that the cells have mesodermal origins. $Ras^{V12}$ line 10 serves as a representative cell line, and its expression of dMef2 is shown (Figure 1A).

The presence of blood cells was tested for with the H2 antibody. H2 recognizes Hemese, a transmembrane protein found on *Drosophila melanogaster* blood cells (hemocytes). Expression of this protein is restricted to the surfaces of all types of hemocytes and hemopoetic organs (Kurucz et al., 2003). $Ras^{V12}$ line 8 showed some positive results when stained with H2 (Figure 1C), however, only a few cells within the culture showed this phenotype.

Finally, D E-Cadherin tests for the presence of epithelial cells by binding to cadherins (calcium dependent adhesion molecules) that are expressed on the cell surface of epithelial cells. Positive results were seen with D E-Cadherin expression in one cell line, $Ras^{V12}$; $wts^{RNAi}$ line E (Figure 1D). Because this line also has an epithelial-like morphology, it is believed to be a true epithelial cell line. This cell line opens up many possibilities for future research in examining polarity. While an epithelial cell line could serve as an excellent system to analyze the Epidermal Growth Factor Receptor pathway *in vitro*, the consitutively active Ras expressed by this line makes this $Ras^{V12}$; $wts^{RNAi}$ line undesirable for such research. However, an additional epithelial line has been created (not shown) with $wts^{RNAi}$ alone, which could be used for Egfr anaylsis.

Although this method has been shown to efficiently generate continuous Drosophila cell lines, it does not allow for the effective production of tissue specific lines. Our lab is currently exploring new techniques to create lines of specific cell types. For instance, our lab is working on the establishment of a muscle cell line using tissue specific induction of Gal4. This would lead to a selective expression of Ras within these tissues, giving these cells a proliferative

advantage within the culture. Also, a technique that would allow for reversible Ras expression is being investigated. With this approach, Ras expression could be used to efficiently establish cell lines, and later be shut off to potentially allow for cell differentiation.

# Chapter 2

## Analysis of the Epidermal Growth Factor Receptor Pathway in *Drosophila melanogaster*

### Background

*The EGF receptor and ligands*

The Epidermal Growth Factor Receptor (Egfr) pathway is a highly conserved pathway that is essential throughout development for many biological processes, such as cell migration, differentiation, proliferation, and tissue patterning. The *Drosophila* Egfr pathway consists of one dimerizing tyrosine kinase receptor and four ligands: Spitz, Keren, Gurken, and Vein. Spitz, Keren, and Gurken are all activating ligands that are produced as transmembrane precursors, while Vein is a secreted activating ligand (see Freeman, 1998; Shilo 2003, 2005, for review).

The primary activating ligand is Spitz, which is ubiquitously produced in an inactive form and retained in the Endoplasmic Reticulum (ER). Activation of Spitz is highly regulated and requires processing from two proteins, Star and Rhomboid (see Schweitzer and Shilo, 1997, for review). Star, which is also ubiquitously expressed, mediates the migration of Spitz from the ER to the Golgi apparatus, where it is cleaved by Rhomboid (Urban et al., 2001). The activated Spitz protein is then transported to the cell surface and secreted. During this process, Rhomboid serves as the limiting agent that regulates activation. Ectopic expression of Rhomboid leads to excess Egfr activity in a wide variety of cell types (Golembo et al., 1996; Sturtevant et al., 1993).

The other two transmembrane ligands, Keren and Gurken, are activated through a similar process. The activation of Gurken is also highly regulated by Star and Rhomboid, but expression of Gurken is limited to the germ cells (see Schweitzer and Shilo, 1997, for review). Keren resembles Spitz, however, the activation of Keren via Star and Rhomboid occurs with much less

regulation. Rhomboid has been observed to cleave Keren in the absence of Star, and overexpression of Keren leads to hyperactive Egfr activity (Reich et al., 2002). All of the transmembrane ligands retain a single EGF domain in their activated form following cleavage by Rhomboid (Schweitzer et al., 1995). This EGF domain serves as the site of ligand-receptor binding (Schnepp et al., 1998).

In comparison with the other activating ligands, less is known about the ligand Vein. As a secreted activating ligand, Vein does not require any post-translational modification to reach an active state (Shilo et al., 2003). In addition to an EGF domain, Vein also contains an immunoglobulin-like domain. The presence of these two domains causes Vein to resemble vertebrate neuroregulins (Schnepp et al., 1996). Also, when compared to Spitz, which is a very potent activator, Vein is a moderate activator of *Drosophila* Egfr signaling (Schnepp et al., 1998) that functions in both the developing embryo and the adult wing (Schnepp et al., 1998).

*Downstream Targets*

Once activating ligands bind to the EGF receptor, the receptors dimerize and cross phosphorylate on Carboxy-terminal tyrosine residues (Schnepp et al. 1998). Activation of these receptors leads to activation of many downstream target proteins. The main route that the signal transduction proceeds is through the Ras/Raf/ERK cascade. The Drosophila ERK *rolled (rl*) activates among many other proteins the transcription factors Pointed P1 and Pointed P2 (Bergmann et al., 1998). Pointed P1 has been shown to activate the Egfr inhibitor *argos*. In this way, *argos* acts in a negative feedback loop to regulate the activity of the Egfr pathway (Klein et al., 2004). The Egfr pathway also has important roles in cell survival. *rl* has been shown to suppress the proapoptotic activity of *head involution defective (hid)* (Bergmann et al., 1997).

*The Drosophila Wing*

The Drosophila wing serves as a good model system for the study of the control of epidermal tissue patterning, shape, and size. The wing is formed from an imaginal wing disc that originates from about 20 cells in the embryo that proliferate throughout the larval stage reaching 50,000 cells in the late 3rd instar. Throughout this proliferation phase, cells receive ordered signaling events and transcriptional regulation that determine the pattern of veins and intervein regions within the wing blade. In *Drosophila melanogaster*, the wing consists of six longitudinal veins (L1-L6) and two transverse veins. (see Crozatier et al., 2004; de Celis, 2003, for review).

Early during development, the cells of the wing disc are divided into two distinct groups, the anterior and the posterior. The anterior population is controlled by the transcription factor Cubitus interruptus (Ci), while the posterior is controlled by the expression of *engrailed (en*). During the late 3rd instar, expression of *blistered (bs)* marks the intervein regions, while the provein regions are marked by the expression of *rhomboid*. The formation of each of the longitudinal veins requires separate signaling mechanisms, which all involve some signaling from the Egfr pathway.

In the center of the wing, *hedgehog (hh)* plays a key role by activating numerous genes. k*not (kn)*, a transcription factor activated by *hh*, activates *bs* in the L4/L3 intervein region and v*ein*, a ligand from the Egfr pathway that is required for the formation of the L4 vein. *hh* is also responsible for activating the *iroquois* gene complex (*iro*), which mediates the expression of the L3 vein by activating the vein determining genes *rhomboid* and *Delta*, which encodes a ligand in the Notch pathway. Positioning of the L2 and L5 veins is influenced by the spalt complex, which is made up of two genes: *spalt* (*sal*) and *spalt-related* (*salr*).

Once the provein regions are established, signaling from both the Egfr and Notch pathways is required for vein formation. Ligands of the Notch pathway, including Delta, are expressed in the provein regions. These ligands activate the Notch pathway in neighboring cells, which results in the prevention of vein formation in these regions. Notch activation also confines the proteins Star and Rhomboid in the provein region, leading to the activation of the Egfr ligands. Egfr signaling prevents the expression of *bs* and allows for vein differentiation. As a result, extra veins and gaps in the veins results from too much and too little Egfr activity, respectively (Schnepp et al., 1996).

*Discovery of New Components in the Egfr Pathway*

The highly conserved Egfr pathway is required for numerous biological mechanisms throughout development; however, many targets of the pathway remain unknown. Through microarray analysis, our lab has discovered several potential targets of the pathway, including *Sulfated (Sulf1)*. *Sulf1* encodes a 6-O-endosulfatase that modifies the glucosamine residues in heparan-sulfated proteoglycans. The vertebrate homolog *HSulf-1* has recently been shown to have been down-regulated in ovarian, breast, and other types of cancer cell lines (Narita et al., 2007).

The role of *Sulf1* and its interactions with the Egfr pathway were analyzed genetically, using the Drosophila wing as a model system. Many stocks used in the screen were created by Jon P. Butchar. These included *Sulf1* overexpression transgenes and *Sulf1* RNAi transgenes, as well as two recombinant lines: *Sulf1 overexpression;en-Gal4* and *Sulf1 RNAi;en-Gal4*. Initial results from Jon P. Butchar indicated that *Sulf1* may act in a negative feedback loop within the Egfr pathway through inhibition of the ligand Vein. My goal was to test these initial findings.

**Methods**

All crosses were carried out at 25°C and 29°C. The following Gal4 and UAS constructs were used: *UAS-Vn1.1 (*Schnepp et al., 1996), *71B-GAL4* (Brand and Perrimon, 1993), A*ctin5C-Gal4 (Act5C-Gal4), engrailed-Gal4 (en-Gal4)* (Aza-Blanc et al., 1997), *optomotor-blind-Gal4 (omb-Gal4)* (Cook et al., 2004), *Sulf1* overexpression lines 1-5, *Sulf1* RNAi line 2, *Sulf1* RNAi line 9, *Sulf1/CG6725* Vienna RNAi 37361, *Sulf1/CG6725* Vienna RNAi 37362, *Sulf1/CG6725* Vienna RNAi 45954. Two recombinants were created by Jon P. Butchar: *Sulf1 overexpression; en-Gal4/Cyo* and *Sulf1 RNAi; en-Gal4/Cyo*.

Virgins of each of the *Sulf1* overexpression lines and the *Sulf1* RNAi lines were crossed with males from all four Gal4-drivers to drive expression of the Sulf1 transgenes. Additionally, *UAS-Vn1.1* virgins were crossed with males from the two recombinants (*Sulf1 overexpression; en-Gal4/Cyo* and *Sulf1 RNAi; en-Gal4/Cyo*) as well as *en-Gal4* in order to analyze the interactions of *Sulf1* and the ligand *vn*.

**Results and Discussion**

The role of *Sulf1* and its interactions with the Egfr ligand *vn* were analyzed with the use of the Drosophila wing as a model system. Because the patterning and differentiation of veins on the wings is partially controlled through the Egfr pathway, induced changes in vein patterning by *Sulf1* could be a result of modulating the Egfr pathway.

*Phenotypic Effects of Sulf1 Knockdown and Overexpression*

Reverse genetics with transgenes was used to disrupt the wildtype functioning of *Sulf1* in order to analyze its role during development. Expression was perturbed by overexpressing a *Sulf1* cDNA (*UAS-Sulf1OE*) and by inhibiting *Sulf1* expression with RNAi (*UAS-Sulf1 RNAi*). The GAL4-UAS system (Brand and Perrimon, 1993) was used to drive the expression of these *Sulf1* transgenes with various drivers: *en-Gal4,* which is expressed in the posterior region of the wing; *omb-Gal4,* which is expressed in the early wing; *71B-Gal4,* which is expressed in the late wing, and *Act5C-Gal4,* which is expressed ubiquitously.

Overexpression of *Sulf1* with *en-Gal4* (*en-Gal4; UAS-Sulf1OE*) gave no additional phenotypes beyond the expression of the *en-Gal4* driver alone. About a quarter of *en-Gal4* flies lose expression of the anterior crossvein (Table 2). Knockdown of *Sulf1* with *Sulf1 RNAi (UAS-Sulf1 RNAi)* appeared to suppress the phenotype seen in *en-Gal4* flies, and additionally, a small fraction also showed an extra vein phenotype (Table 2).

*Sufl1* overexpression (*UAS-Sulf1OE*) and knockdown of *Sulf1* (*UAS-Sulf1 RNAi*) also showed no extra phenotypes with *omb-Gal4* than those seen with expression of the *omb-Gal4* activator with *yw.* Flies expressing *omb-Gal4* showed extra veins, as well as a loss of tissue at the posterior end of the wing (Table 2).

Additionally, overexpression of *Sulf1* (*UAS-Sulf1OE*) again showed no extra phenotypes with *71B-Gal4* other than those seen with expression of the *71B-Gal4* driver alone. Flies expressing *71B-Gal4* were observed to have extra anterior crossveins (Table 2). Knockdown of *Sulf1* with *Sulf1 RNAi* expressed with *71B-Gal4 (71B-Gal4; UAS-Sulf1 RNAi)* seemed to suppress the extra vein phenotype (Table 2).

Currently, analysis of *Sulf1* overexpression (*UAS-Sulf1OE*) and knockdown of *Sulf1 (UAS-Sulf1 RNAi)* with A*ct5C-Gal4* is still in progress.

| **Genotype** | **% Viability** | **Observed Phenotype** | **% with Phenotype** |
|---|---|---|---|
| *en-Gal4; Sulf1RNAi* | 100 | Extra veins | 8 |
| *en-Gal4; Sulf1 OE* | 100 | Missing anterior crossvein | 14 |
| *en-Gal4* | 100 | Missing anterior crossvein | 25 |
| *omb-Gal4; Sulf1 RNAi* | 92 | Loss of tissue and extra veins | 84 |
| *omb-Gal4; Sulf1 OE* | 78 | Loss of tissue and extra veins | 75 |
| *omb-Gal4; yw* | 100 | Loss of tissue and extra veins | 100 |
| *71B-Gal4; Sulf1 RNAi* | 100 | No phenotype | NA* |
| *71B-Gal4; Sulf1 OE* | 100 | Extra anterior crossveins | 10 |
| *71B-Gal4* | 100 | Extra anterior crossveins | 18 |
| *Act5C-Gal4; Sulf1 RNAi* | In Progress | | |
| *Act5C-Gal4; Sufl1 OE* | In Progress | | |
| *Act5C-Gal4/ TM6,Tb,Hu* | In Progress | | |

**Table 2. RNAi and overexpression of *Sulf1*.** *Sulf1* overexpression with *en-Gal4, omb-Gal4,* and *71B-Gal4* showed no additional phenotypes beyond the expression of the drivers alone. *Sulf1 RNAi* with *omb-Gal4* also did not show extra phenotypes other than those observed with expression of *omb-Gal4* with *yw.* Knockdown of *Sulf1* with *Sulf1 RNAi* expressed with *en-Gal4* and *71B-Gal4* appeared to suppress the phenotype seen in expression of the drivers alone, and additionally, *Sulf1 RNAi* with *en-Gal4* showed an extra vein phenotype. Currently, analysis with the ubiquitous driver Act5C is still in progress.
*NA not applicable

These results indicate that the initial findings of Jon P. Butchar could not be substantiated when the analysis was expanded to include additional lines of *Sulf1* overexpression (*UAS-Sulf1OE*) and *Sulf1 RNAi (UAS-Sulf1 RNAi)*. After analyzing the controls, many of the phenotypes that were initially thought to be the result of *Sulf1* were shown to be caused by expression of the Gal4 drivers alone.

*Interactions between Sulf1 and Vein*

The interactions between *Sulf1* and *vn* were analyzed through genetic tests. Lethality in the pupal stage is observed when the UAS-regulated construct of *vn* (*UAS-Vn1.1*) was under the control of the driver *en-Gal4* (Fig. 3). However, Jon P. Butchar reported that when the flies also overexpressed Sulf1, they were rescued. Although the flies were rescued, they were not restored to wildtype due to the extra vein and blisters phenotype that was observed (Fig. 3). I am currently repeating these crosses.

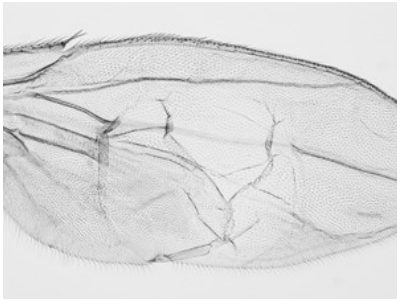
| | Control (*en*-Gal4) | *Sulf1* RNAi; en-Gal4 | *Sulf1* overexpression; en-Gal4 |
|---|---|---|---|
| UAS-*Vn1.1* | Lethal | Lethal |  |

**Figure 3. Interactions of *Sulf1* and V*ein*.** Figure by Jon P. Butchar. Expression of UAS-Vn1.1 driven by *en*-Gal4 as well as *Sulf1 RNAi; en-Gal4* result in lethality at 25°C. Overexpression of *Sulf1* rescues this lethality, but the wings were not wildtype as extra veins and blisters were observed.

*Future Directions*

If I can confirm the rescue of *Vn1.1; en-Gal4* with overexpression of *Sulf1*, future studies could analyze how Sulf1 and Vn1.1 physically interact. One possible mechanism for the interaction between *Sulf1* and *vn* is through the Ig-like domain of Vein. In vertebrates, it has been demonstrated that the Ig-like domain of neuregulins, such as neuregulin-1, is involved in their binding to heparan-sulfated proteoglycans (Pankonin, 2005). This suggests that Vein may bind to heparan-sulfated proteoglycans through its Ig-like domain, and the strength of this interaction may be regulated by the ability of Sulf1 to remove the 6-O sulfate from the proteoglycans.

**References**


Acar, M., Jafar-Neiad, H., Takeuchi, H., Rajan, A., Ibrani, D., Rana, N. A., Pan, H., Haltiwanger, R. S., Bellen, H. J. (2008). Rumi is a CAP10 domain glycosyltransferase that modifies Notch and is required for Notch signaling. *Cell*, 132(2): 247-258.

Aza-Blanc, P, et al. (1997). Proteolysis that is inhibited by hedgehog targets Cubitus interruptus protein to the nucleus and converts it to a repressor. *Cell*, 89(7): 1043-53.

Banker, G. A., Cowan, W. M. (1977). Rat hippocampal neurons in dispersed cell culture. *Brain Res*., 126(3): 397-42.

Bergmann, A., Agapite, J., McCall, K., Steller, H. (1998). The Drosophila gene *hid* is a direct molecular target of Ras-dependent survival signaling. *Cell*, 95: 331-341.

Brand, A. H., and Perrimon, N. (1993). Target gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, 118(2): 401-15.

Cook, O., Biehs, B., Bier, E. (2004). brinker and optomotor-blind act coordinately to initiate development of the L5 wing vein primordium in Drosophila. *Development*, 131(9): 2113-2124.

Crozatier, M., Glise, B., and Vincent, A. (2004). Patterns in evolution: veins of the Drosophila wing. *Trends Genet,* 20(10): 498–505.

de Celis, J. F. (2003). Pattern formation in the Drosophila wing: The development of the veins. *Bioessays*, 25(5): 443–451.

Debec, A., (1978). Haploid cell cultures of Drosophila melanogaster. *Nature*, 274: 255-256.

Foddis, R., De Rienzo, A., Broccoli, D., Bocchetta, M., Stekala, E., Rizzo, P., Tosolini, A., Grobelny, J. V., Jhanwar, S. C., Pass, H. I., Testa, J. R., Carbone, M. (2002). SV40 infection induces telomerase activity in human mesothelial cells. *Oncogene*, 21: 1434-1442. Doi: 10.1038/sj/onc/1205203.

Freeman, M. (1998). Complexity of EGF receptor signalling revealed in Drosophila. *Curr Opin Genet Dev*, 8(4): 407–411.

Golembo, M., Raz, E. and Shilo, B. Z. (1996). The Drosophila embryonic midline is the site of Spitz processing, and induces activation of the EGF receptor in the ventral ectoderm. *Development,* 122: 3363-3370.

Howlin, J., Rosenkvist, J., Andersson, T. (2008). TNK2 preserves epidermal growth factor receptor expression on the cell surface and enhances migration and invasion of human

breast cancer cell. *Breast Cancer Research*, 10:R36. Doi:10.1186/bcr2087.

Huang, Q., Chen, M., Liang, S., Acha, V., Liu, D., Yuan, F., Hawks, C. L., Hornsby, P. J. (2007). Improving cell therapy-experiments using transplanted telomerase-immortalized cells in immunodeficient mice. *Mech Ageing Dev.*, 128(1): 25-30.

Klein, D. E., Nappi, V. M., Reeves, G. T., Shvartsman, S. Y., Lemmon, M. A. (2004). Argos inhibits epidermal growth factor receptor signalling by ligand sequestration. *Nature*, 430(7003): 1040-1044.

Korochkin, L., Saveliev, S., Ivanov, A., Evgeniev, M., Bessova, N., Gulimova, V. (1991). Nerve cells of Drosophila Notch mutant are differentiated inside amphibian brain: a new approach for the analysis of genetic control of nerve cell differentiation. *Genetica*, 85: 23-34.

Kuiyama, M., Harada, N., Kuroda, S., Yamamoto, T., Nakafuku, M., Iwamatsu, A., Yamamoto D., Prasad, R., Croce, C., Canaani, E., Kaibuchi, K.. (1996). Identification of AF-6 and canoe as putative targets for Ras. *J Biol Chem*, 271(2): 607-610.

Kurucz, E., Zettervall, C.J., Sinka, R., Vilmos, P., Pivaicsi, A., Ekengren, S., Hegedus, Z., Ando, I., Hultmark, D. (2003). Hemese, a hemocyte-specific transmembrane protein, affects the cellular immune response in Drosophila. P*roc Nad Acad Sci*, 100(5): 2622-2627.

Li, A. F., Broad, S., Lu, Y. J., Yang, J. S., Watson, R., Hagemann, T., Wilbanks, G., Jacobs, I., Balkwill, F., Dafou, D., Gayther S. A. (2007) Human ovarian surface epithelial cells immortalized with hTERT maintain functional pRb and p53 expression. *Cell Proliferation*, 40(5): 780-794.

Lilly, B., Zhao, B., Ranganayakulu, G., Paterson, B.M., Schulz, R.A., Olson, E. N. (1995). Requirement of MADS domain transcription factor D-MEF2 for muscle formation in Drosophila. *Science*, 267: 688-693.

Meuillet, E. J., Mania-Farnell, B., George, D., Inokuchi, J. I., Bremer, E. G. (2000). Modulation of EGF receptor activity by changes in the GM3 content in human epidermoid carcinoma cell line, A431. *Exp Cell Res.*, 256(1): 74-82.

Narita, K., Chien, J., Mullany, S. A., Staub, J., Qian, X., Lingle, W. L., Shridhar, V. (2007). Loss of HSulf-1 expression enhances autocrine signaling mediated by amphiregulin in breast cancer. *J Biol Chem*, 282(19): 14413-14420.

Pankonin, M. S., Gallagher, J. T., Loeb, J. A. (2005). Specific structural features of heparan sulfate proteoglycans potentiate Neuregulin-1 signaling. *J Biol Chem*, 280(1): 383-388.

Reich, A., Shilo, B.Z., Keren. (2002). A new ligand of the *Drosophila* epidermal growth factor

receptor, undergoes two modes of cleavage. *EMBO J.,* 21: 4287–4296.

Schneider, I. (1972). Cell lines derived from late embryonic stages of *Drosophila melanogaster*. *J Embryol. Exp. Morph.*, 27(2): 353-365.

Schnepp, B., Donaldson, T., Grumbling, G., Ostrowski, S., Schweitzer, R., Shilo, B. Z., and Simcox, A. (1998). EGF domain swap converts a drosophila EGF receptor activator into an inhibitor. *Genes Dev*, 12(7): 908–913.

Schnepp, B., Grumbling, G., Donaldson, T., and Simcox, A. (1996). Vein is a novel component in the Drosophila epidermal growth factor receptor pathway with similarity to the neuregulins. *Genes Dev*, 10 (18): 2302–2313.

Schweitzer, R., Howes, R., Smith, R., Shilo, B. Z., and Freeman, M. (1995). Inhibition of Drosophila EGF receptor activation by the secreted protein Argos. *Nature*, 376(6542): 699–702.

Schweitzer, R., Shilo, B. Z. (1997). A thousand and one roles for the Drosophila EGF receptor. *Trends in Genetics*, 13(5): 191-196.

Shilo, B. Z. (2003). Signaling by the Drosophila epidermal growth factor receptor pathway during development. *Exp Cell Res*, 284(1): 140–149.

Shilo, B.-Z. (2005). Regulating the dynamics of EGF receptor signaling in space and time. *Development*, 132(18): 4017–4027.

Simcox, A. A., Sobeith, M. M., Shearn, A. (1985). Establishment and characterization of continuous cell lines derived from temperature-sensitive mutants of Drosophila melanogaster. *Somatic Cell and Molecular Genetics*, 11: 63-70.

Simcox, A., Mitra, S., Truesdell, S., Paul, L., Chen, T., Butchar, J. P., Justiniano, S. (2008a). Efficient Genetic Method for Establishing Drosophila Cell Lines Unlocks the Potential to Create Lines of Specific Genotypes. *PloS Genet,* 4(8): e1000142. Doi:10.1371/journal.pgen.1000142.

Simcox, A. A., Austin, C. L., Jacobsen, T. L., Jafar-Nejad, H. (2008b). Drosophila embryonic 'fibroblasts': extending mutant analysis in vitro. *Fly*, 2(6): 306-309.

Stanley, P. (2008). Glucose: A Novel Regulator of Notch Signaling. *ACS Chemical Biology*, 3(4): 210-213.

Sturtevant, M. A., Roark, M. and Bier, E. (1993). The Drosophila rhomboid gene mediates the localized formation of wing veins and interacts genetically with components of the EGF-R signaling pathway. *Genes Dev,* 7: 961-973.

Urban, S., Lee, J.R., Freeman, M. (2001). *Drosophila* rhomboid-1 defines a family of putative intramembrane serine proteases. *Cell*, 107: 173–182.

Yang, G., Rosen, D. G., Mercado-Uribe, I., Colacino, J. A., Mills, G. B., Bast Jr., R. C., Zhou, C., Liu, J. (2007). Knockdown of *p53* combined with expression of the catalytic subunit of telomerase is suffiecient to immortalize primary human ovarian surface epithelial cells. *Carcinogenesis*, 28(1): 174-182.